Synthesis, Fungicidal Activity, and Effects on Fungal Polyamine Metabolism of Novel Cyclic Diamines

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A number of novel, cyclic diamines were synthesized and examined for fungicidal activity as part of a continuing program of work on polyamine analogues. The novel synthetic cyclic diamines *trans*-1,2-bis(diethylaminomethyl)cyclopentane (compound **1**) and *trans*-5,6-bis(aminomethyl)bicyclo[2.2.1]-hept-2-ene (compound **2**) and the synthetic cyclic diamine 1,2-bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene (compound **3**) controlled the important crop pathogen *Erysiphe graminis* DC f.sp. *hordei* Marchal. Since *E. graminis* cannot be cultured *in vitro*, the effects of the three diamines on polyamine biosynthesis were studied using the fungal pathogen *Pyrenophora avenae* Ito & Kuribay. All three compounds were effective in reducing the growth of *P. avenae in vitro* and in altering polyamine levels. However, whereas compound **1** reduced concentrations of all three polyamines, compound **2** increased spermidine 2-fold and compound **3** had little effect on spermidine and spermine concentrations but reduced putrescine concentration by 69%. These changes in polyamine concentrations could not be correlated with changes in activities of biosynthetic enzymes. It seems therefore that although these novel cyclic diamines alter fungal polyamine metabolism, their effects on the growth of *P. avenae* may not be related to depletion of cellular polyamines.

Keywords: *Polyamines; cyclic diamines; fungicide; powdery mildew*

INTRODUCTION

Polyamine biosynthesis inhibitors can give effective control of biotrophic fungal pathogens, such as rusts and powdery mildews (Rajam *et al*., 1985; Walters, 1986; West and Walters, 1988). Much of this early work used enzyme-activated irreversible inhibitors, e.g. α-difluoromethylornithine (DFMO), an inhibitor of the polyamine biosynthetic enzyme ornithine decarboxylase (ODC) (Metcalf *et al*., 1978). Indeed, DFMO has been shown to control a range of fungal pathogens in glasshouse experiments and to control powdery mildew on barley in field trials (Walters *et al*., 1992). Since powdery mildew, for example, cannot be grown axenically, it has been difficult to determine whether DFMO-induced reductions in mildew infection are due to alterations in polyamine biosynthesis. Nevertheless, DFMO was shown to deplete intracellular concentrations of putrescine and spermidine in the necrotrophic pathogen *Pyrenophora avenae* (Foster and Walters, 1990). More recently, an alternative method of polyamine perturbation has been demonstrated using polyamine analogues (Porter and Sufrin, 1986). As a result, a variety of polyamine analogues have been shown to alter polyamine metabolism in tumor cells leading to pronounced antiproliferative effects. In recent work, ketoputrescine, a commercially available putrescine analogue, was shown to possess fungicidal properties (Foster and Walters, 1993). In previous papers, we have described the fungicidal activity of a number of aliphatic putrescine analogues (Havis *et al*., 1994a,b). This paper describes the synthesis, as their salts and free bases, fungicidal evaluation, and effects on polyamine metabolism in *P. avenae* of a number of novel cyclic diamines.

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EXPERIMENTAL METHODS

Synthesis of *trans*-1,2-Bis(diethylaminomethyl)cyclopentane (1). *trans*-1,2-Cyclopentanedicarboxylic acid (0.5 g, 3.16 mmol) was heated under reflux with thionyl chloride (6 equiv) for 6 h. The excess thionyl chloride was then removed under reduced pressure. The crude acid chloride was dissolved in ether (5 mL) and was added dropwise to a stirred solution of diethylamine (3.24 mL, 34 mmol, 10 equiv) in ether (10 mL) cooled to 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The precipitated diethylamine hydrochloride was filtered off and extracted several times with ether. The combined organic extracts were concentrated *in vacuo* to give *trans*-*N*,*N*,*N*′,*N*′-tetraethylcyclopentane-1,2-dicarboxamide as a light yellow oil (0.73 g, 89%): ^{1}H NMR (90 MHz, $CDCl_3$) 1.13 (12H, m), 1.75 (4H, m), 2.00 (2H, m), and 3.20−3.55 ppm (8H and 2H, m).

To a solution of lithium aluminum hydride (0.45 g, 11.9 mmol) in dry ether (5 mL), under an atmosphere of nitrogen, was added dropwise a solution of *trans*-*N*,*N*,*N*′,*N*′-tetraethylcyclopentane-1,2-dicarboxamide (0.8 g, 2.98 mmol) in dry ether (5 mL). When the addition was complete, the mixture was heated at reflux for 1 h. The mixture was then cooled in an ice bath, and excess hydride was decomposed by the dropwise addition of water (1 mL), followed by a 15% solution of sodium hydroxide (1 mL) and then water (2 mL) again. After 10 min of vigorous stirring, the mixture was filtered with suction and the granular precipitate was washed thoroughly with ether. The filtrate was dried ($MgSO_4$) and concentrated *in vacuo* to give *trans*-1,2-bis(diethylaminomethyl)cyclopentane as a light colored oil (0.71 g, 87%): IR (thin film) 2968, 2936, 2871, and 2796 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) 0.99 (12H, t), 1.31 (2H, m) 1.46−1.82 (4H and 2H, m), 2.32 (4H, m), and 2.48 ppm (8H, q); ^{13}C NMR (50 MHz) 11.5 (CH_3), 24.2 (CH_2), 31.1 (CH_2), 41.9 (CH), 47.0 (CH_2), and 58.7 ppm (CH_2); MS, *m*/*z* 241 (M^+ + 1), 240 (M^+, 7.7%), 211, 152, 138, 112, 99, 86 (100%), 72,

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and 58. (Found: C, 74.84; H, 13.10; N, 11.79; M^+, 240.2564. $C_{15}H_{32}N_2$ requires C, 74.91; H, 13.42; N, 11.66; M^+, 240.2565.)

Synthesis of *trans*-5,6-Bis(aminomethyl)bicyclo[2.2.1]-hept-2-ene (2). To a cooled solution of fumaronitrile (2.73 g, 35 mmol) in ethanol (20 mL) was added dropwise, with stirring, freshly distilled cyclopentadiene (2.55 g, 38 mmol). When the addition was complete, the solution was concentrated *in vacuo* to half its original volume. The solution was cooled to 0 °C, and an ice crystal was used to seed crystallization. The product was recrystallized from ethanol to afford *trans*-bicyclo[2.2.1]hept-2-ene-5,6-dinitrile (3.81 g, 75%): mp 92−94 °C [Blomquist and Winslow (1945) mp 95.5−96 °C]; IR (KBr disc) 3448, 3072, 2998, and 2242 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 1.64−1.81 (2H, m), 2.50−2.53 (1H, m), 3.16−3.19 (1H, m), 3.40−3.45 (2H, m), and 6.37 ppm (2H, m); ^{13}C NMR (50 MHz) 34.5 (CH), 34.6 (CH), 46.2 (CH), 47.2 (CH), 48.2 (CH), 119.5 (CH), 119.9 (CH), 135.6 (CH), and 137.1 ppm (CH); MS, *m/z* 144 (M^+, 0.3%), 117, 104, 90, 77, 66 (100%), 51, and 39. (Found: C, 75.10; H, 5.45; N, 19.44; M^+, 144.0674. $C_9H_8N_2$ requires C, 75.00; H, 5.55; N, 19.44; M^+, 144.0688.)

A three-neck flask was equipped with stopper, septum, and condenser with nitrogen balloon. Lithium aluminum hydride (1.06 g, 27.9 mmol) was put into the flask, and dry ether (15 mL) was added. *trans*-Bicyclo[2.2.1]hept-2-ene-5,6-dinitrile (1 g, 6.94 mmol) in dry ether (25 mL) was added dropwise over 20 min. Toward the end of the reaction the mixture became thick and difficult to stir. After addition was complete, the reaction was chilled to 0 °C and water (2 mL), 15% sodium hydroxide solution (2 mL), and more water (5 mL) were added sequentially and cautiously. The resulting white precipitate was filtered off and extracted with dry ether (3 × 20 mL). The ether extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give trans-5,6-bis(aminomethyl)bicyclo[2.2.1]hept-2-ene as a colorless oil (0.98 g, 93%): IR (thin film) 3291, 2959, 2909, 2870, 2432, 1570, and 1458 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 0.90 (1H, ddd), 1.36 (1H, d), 1.44 (1H, d), 1.60 (1H, ddd), 2.26 (1H, dd), 2.45 (1H, dd), 2.57 (1H, dd), 2.61 (1H, bs), 2.73 (1H, dd), 2.84 (1H, bs), 6.06 (1H, dd), and 6.23 ppm (1H, dd); ^{13}C NMR (50 MHz) 44.6 (CH), 45.5 (CH), 46.0 (CH_2), 46.9 (2 × CH_2), 47.7 (CH), 48.3 (CH), 135.0 (CH), and 138.0 ppm (CH); MS, *m/z* 153 ($M^+ + 1$), 152 (M^+, 1.0%), 135, 122, 106, 91, 78, 69, 66 (100%), and 56. (Found: M^+, 152.1314. $C_9H_{16}N_2$ requires M^+, 152.1314.)

Synthesis of 1,2-Bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene Dihydrochloride (3). 2,3-Dimethyl-1,3-butadiene (1.64 g, 20 mmol), dimethyl acetylenedicarboxylate (2.84 g, 20 mmol), and water (50 mL) were heated at 60 °C for 24 h. The reaction mixture was then cooled, and the precipitate was filtered off and washed with water. The white solid was recrystallized from acetone to give colorless crystals of dimethyl 4,5-dimethylcyclohexa-1,2-diene-1,2-dicarboxylate (4.48 g, 73%): IR (KBr disc) 3446, 2956, 2859, 1740, 1724, and 1696 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 1.66 (6H, s), 2.92 (4H, s), and 3.77 ppm (6H, s); ^{13}C NMR (50 MHz) 17.8 (CH_3), 33.9 (CH_2), 52.0 (CH_3), 121.4 (C), 132.6 (C), and 168.2 ppm (CO); MS, *m/z* 225 ($M^+ + 1$), 244 (M^+, 4.6%), 191, 177 (100%), 133, 105, 91, 77, and 59. (Found: C, 64.13; H, 7.02; M^+, 224.1055. $C_{12}H_{16}O_4$ requires C, 64.25; H, 7.19%; M^+, 224.1049.)

Dimethyl 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylate (2 g, 8.9 mmol) was dissolved in methanol (5 mL), and lithium hydroxide monohydrate (2 g, 47.6 mmol) in methanol/water (3:1) (40 mL) was added slowly. A yellow color appeared, and stirring was continued for 24 h. After this time, the yellow color disappeared and the mixture was washed with ether (3 × 20 mL) to remove any unreacted diester. Hydrochloric acid (3 M, 10 mL) was added dropwise, and a cloudy suspension appeared. Another portion of 3 M hydrochloric acid (5 mL) was added dropwise, and the solution went clear (pH 2) and was extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate extracts were dried (Na_2SO_4) and concentrated *in vacuo* to give a white solid of 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (1.00 g, 57%): IR (KBr disc) 3421, 2935, 2632, and 1694 cm^{-1}; ^1H NMR (200 MHz, D_2O and DMSO) 1.59 (6H, s) and 2.84 ppm (4H, s); ^{13}C NMR (50 MHz) 18.2 (CH_3), 34.5 (CH_2), 122.7 (C), 133.8 (C), and 172.8 ppm (CO); MS, *m/z* 197 ($M^+ + 1$), 196 (M^+, 10.8%), 178, 151, 133, 107, 91 (100%), 77, and 51. (Found: M^+, 196.0732. $C_{10}H_{12}O_4$ requires M^+, 196.0735 DMSO-d_6.)

To a suspension of 4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxylic acid (1 g, 5.1 mmol) in benzene (10 mL) was added hexamethylphosphorous triamide (0.52 mL, 5.1 mmol) dropwise. The mixture was reheated at reflux for 20 min and then allowed to cool to room temperature. Saturated sodium bicarbonate solution (10 mL) was added, and the layers were separated. The aqueous layer was extracted with dichloromethane (3 × 20 mL), and the extracts were combined, dried ($MgSO_4$), and concentrated *in vacuo* to give a yellow oil (0.63 g). The ^1H NMR spectrum contained *N,N,N′,N′*-tetramethyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboxamide (with some starting material): ^1H NMR (90 MHz, $CDCl_3$) 1.75 (6H, m), 2.30−2.80 (4H, m), 2.95 (6H, s), and 3.10 ppm (6H, s).

To a solution of lithium aluminum hydride (0.36 g, 9.47 mmol) in dry ether under an atmosphere of nitrogen was added dropwise a solution of *N,N,N′,N′*-tetramethyl-4,5-dimethylcyclohexa-1,4-diene-1,2-dicarboximide (0.6 g, 2.4 mmol) in dry ether (5 mL). When the addition was complete, the mixture was heated at reflux for 1 h. The mixture was then cooled in an ice bath, and excess hydride was decomposed by the dropwise addition of water (0.5 mL), followed by 15% sodium hydroxide solution (1 mL) and then water (*ca.* 2 mL) again. After 10 min of vigorous stirring, the mixture was filtered with suction. The granular precipitate was washed thoroughly with ether (3 × 20 mL), and the filtrate was dried ($MgSO_4$) and concentrated *in vacuo* to give 1,2-bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene as an oil (0.12 g, 53%): IR (thin film) 3802, 3422, 2918, 2866, 2523, and 1468 cm^{-1}; ^1H NMR (200 MHz, D_2O) 1.29 (3H, s), 1.32 (3H, s) 2.00−2.34 (4H, m), 2.15 (6H, s), 2.16 (6H, s), and 2.71−3.02 ppm (4H, m); ^{13}C NMR (50 MHz) 17.2 (CH_3), 19.5 (CH_3), 34.8 (CH_2), 43.2 (CH_3), 44.4 (CH_3), 62.2 (CH_2), 63.6 (CH_2), 123.9 (C), and 129.1 (C); MS, *m/z* 223 ($M^+ + 1$−2HCl), 222 (M^+ − 2HCl, 3.8%), 164, 119, 105, 91, 77, 58 (100%), 42, and 30. (Found: M^+ − 2HCl, 222.2108. $C_{14}H_{26}N_2$ requires M^+ −2HCl, 222.2096.)

Determination of the Fungicidal Activity of Cyclic Compounds. The analogues were applied to plants, and fungicidal activity was determined as described in detail previously (Havis *et al*., 1994a). Briefly, the protectant and curative activity of compounds **1**−**3** was studied using barley infected with the powdery mildew fungus, *Erysiphe graminis* f.sp. *hordei*, i.e. treatments were made 3 h preinoculation and 3 days postinoculation. In addition, the curative activity of compounds **1**−**3** was examined using broad bean infected with rust, *Uromyces viciae-fabae* (Pers.) Schroet, or chocolate spot, *Botrytis fabae* Sardina. Inhibitors were applied to plants as aqueous solutions in 0.01% Tween 20. Figures for percentage leaf area infected on barley are the means of 20 replicates. All experiments were repeated twice.

Effects of Compounds 1−3 on Growth, Enzyme Activities, and Polyamine Concentrations in *P. avenae*. The effects of compounds **1**−**3** on mycelial growth, polyamine concentrations, and activities of ornithine decarboxylase (ODC; EC 4.1.1.17) and *S*-adenosylmethionine decarboxylase (AdoMet-DC; EC 4.1.1.50) were determined as described previously (Foster and Walters, 1990; Havis *et al*., 1994a). Fungal tissue was exposed to the diamines in liquid culture for 3 days. Because of the very limited quantities of the compounds available, it was not possible to expose the fungus to the compounds for various periods of time.

RESULTS AND DISCUSSION

Three novel cyclic diamines have been shown to possess substantial fungicidal activity against *E. graminis* on barley, when applied pre- or postinoculation. Best control of *E. graminis* infection was achieved using compound **1** (Figure 1), applied as a postinoculation treatment (96%; Table 1). This compares favorably with the level of disease control achieved with the commercial fungicide propiconazole (98%; Table 1). The superior control obtained with the commercial standard could be

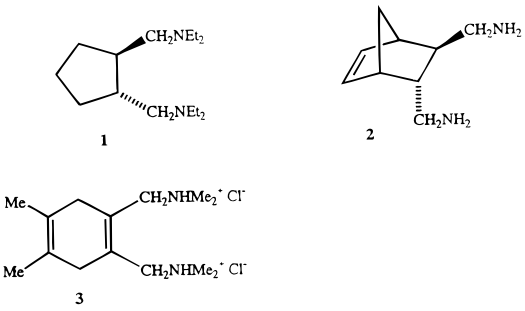

Figure 1. Chemical structures of cyclic diamines.

Table 1. Effect of Cyclic Compounds on Infection of Barley with the Powdery Mildew Fungus, *E. graminis* f.sp. *hordei*

	postinoculation treatment		preinoculation treatment	
treatment[a]	leaf area infected (%)	disease control (%)	leaf area infected (%)	disease control (%)
control	34 ± 2.7		76 ± 2.1	
compound **1**	1 ± 0.3[b]	96	67 ± 1.9[c]	12
compound **2**	21 ± 2.3[c]	36	37 ± 3.4[b]	52
compound **3**	12 ± 2.3[b]	63	43 ± 3.1[b]	43
propiconazole	0.7 ± 0.2[b]	98		

[a] Compound **3** was used as the dihydrochloride salt and was applied at 1.0 mM (i.e. 295 mg L^{-1}), and compounds **1** and **2** were used as free bases and applied at 1.0 mM (i.e. 240 and 152 mg L^{-1}, respectively). Compound **1**, *trans*-1,2-bis(diethylaminomethyl)cyclopentane; compound **2**, *trans*-5,6-bis(aminomethyl)bicyclo-[2.2.1]hept-2-ene; compound **3**, 1,2-bis(dimethylaminomethyl)-4,5-dimethylcyclohexa-1,4-diene dihydrochloride. [b,c] Significantly different from the control at $P \leq 0.001$ and $P \leq 0.005$, respectively; [d] Propiconazole was used as a 0.1% solution (i.e. 250 mg L^{-1}).

related to its formulation, since compound **1** was applied as a simple aqueous solution with 0.01% Tween 20, and formulation is known to improve the efficacy of fungicidal compounds. For compounds **1** and **3**, postinoculation treatments were more effective than preinoculation treatments (Table 1). This effect was also observed with the synthetic putrescine analogues, E-BED and E-TED (Havis *et al*., 1994a,b) and the novel, alicyclic diamine BAD (Robins and Walters, 1993). This effect could be related to perturbation of polyamine biosynthesis in the germinating conidia on the leaf surface. An inhibitor of ODC, DFMO, has previously been shown to inhibit the germination of rust uredospores on the leaf surface (Rajam *et al*., 1989). More recently, DFMO, as well as the putrescine analogues E-BED and E-TED, were shown to inhibit appressorium formation in the rust fungus *U. viciae-fabae* (Reitz et al., 1995). Indeed, it has been suggested that inhibitors of polyamine biosynthesis exert their main effect against early fungal development on the leaf surface (Walters, 1995).

Compounds **1**−**3** also exhibited limited activity against low levels of *B. fabae* infection on broad bean when applied as postinoculation treatments and gave poor control of *U. viciae-fabae* on the same host (data not shown). These results are markedly different from those obtained with the aliphatic putrescine analogues, E-BED and E-TED, both of which gave significant control of *U. viciae-fabae* when applied as a postinoculation treatment (Havis et al., 1994a,b). The reasons for this difference are not known.

Since powdery mildew cannot be grown axenically, the effects of the three novel cyclic diamines on polyamine biosynthetic enzymes and polyamine levels were examined in the oat stripe pathogen, *Pyrenophora avenae*. Interestingly, while all of the cyclic diamines reduced growth of the fungus significantly (Table 2), each produced different effects on polyamine levels and enzyme activities.

Table 2. Effect of Cyclic Compounds on the Growth of *P. avenae* in Liquid Culture

treatment[a]	mean wt (g)	treatment[a]	mean wt (g)
control	1.20 ± 0.02	control	3.07 ± 0.30
compound **1**	0.91 ± 0.03[b]	compound **3**	1.26 ± 0.22[c]
compound **2**	0.87 ± 0.04[c]		

[a] Compound **3** was used as the dihydrochloride salt and was applied at 1.0 mM (i.e. 295 mg L^{-1}); compounds **1** and **2** were used as the free bases and applied at 1.0 mM (i.e. 240 and 152 mg L^{-1}, respectively). [b,c] Significantly different from the control at $P \leq 0.001$ and $P \leq 0.01$, respectively.

Table 3. Effect of Cyclic Compounds on Polyamine Concentrations in *P. avenae*

	polyamine concentration (mol g^{-1} fresh wt^{-1})		
treatment[a]	putrescine	spermidine	spermine
control	136.3 ± 9.9	129.3 ± 16.0	260.9 ± 12.6
compound **1**	21.9 ± 4.0[b]	57.9 ± 2.4[c]	93.6 ± 8.4[b]
compound **2**	71.6 ± 9.7[c]	262.8 ± 15.2[b]	118.9 ± 12.1[b]
compound **3**	43.4 ± 18.6[c]	129.9 ± 24.0	229.4 ± 41.5

[a] Compound **3** was used as the dihydrochloride salt and applied at 1.0 mM (i.e. 295 mg L^{-1}); compounds **1** and **2** were used as the free bases and applied at 1.0 mM (i.e. 240 and 152 mg L^{-1}, respectively). [b,c] Significantly different from the control at $P \leq 0.001$ and $P \leq 0.01$, respectively.

Table 4. Effect of Cyclic Compounds on Enzyme Activities in *P. avenae*

	enzyme activity [pmol of CO_2 (mg of protein)$^{-1}$ h^{-1}]	
treatment[a]	ODC	AdoMetDC
control	259.5 ± 20.7	53.7 ± 19.0
compound **1**	170.4 ± 9.0[b]	77.8 ± 21.4
compound **2**	415.9 ± 28.5[c]	49.9 ± 8.0
compound **3**	211.3 ± 10.4	140.4 ± 35.1

[a] Compound **3** was used as the dihydrochloride salt and was applied at 1.0 mM (i.e. 295 mg L^{-1}); compounds **1** and **2** were used as the free bases and applied at 1.0 mM (i.e. 240 and 152 mg L^{-1}, respectively). [b,c] Significantly different from the control at $P \leq 0.001$ and $P \leq 0.005$, respectively.

Compound **1** produced significant reductions in the levels of putrescine, spermidine, and spermine (84%, 55%, and 64%, respectively; Table 3). The decrease in putrescine concentration can be partly attributed to the significant decrease in ODC activity observed in the fungus, although, despite a small, insignificant increase in AdoMetDC activity (Table 4), spermidine and spermine levels were reduced. These reductions could be related to the lower putrescine levels available for conversion into higher polyamines.

Treatment with compound **2** led to significant reductions in the cellular concentrations of putrescine and spermine (47% and 54%, respectively), while spermidine levels were doubled in the fungal cells (Table 3). These changes were accompanied by a significant increase in ODC activity (60%; Table 4), which should have led to an increase in putrescine levels in the cells. The observed reduction in putrescine concentration may be related to increased putrescine catabolism or increased putrescine efflux from *P. avenae* grown in the presence of compound **2**, neither of which was examined in the present work because of limited supplies of the test compounds. The increased spermidine levels observed are more difficult to explain, especially as AdoMetDC activity was unaltered. It is known that spermidine can be formed from acteylspermine in fungi (Large, 1992).

Recent work has shown that changes in acetylpolyamines occur in *P. avenae* exposed to inhibitors of spermidine biosynthesis (Mackintosh and Walters, unpublished results). It would be useful, therefore, to examine changes in acetylspermine in *P. avenae* exposed to compound **2**, although this will require further synthesis of the compound. Clearly, further work is needed to elucidate the precise mechanism responsible for the observed changes in polyamines in fungal tissue exposed to compound **2** and to determine whether the observed changes in polyamines are involved in the antifungal activity of this compound.

Compound **3** reduced putrescine concentration in *P. avenae* by 68% but had no effect on spermidine or spermine concentrations (Table 3). The reduction in putrescine was not accompanied by a significant decrease in ODC activity (Table 4). This would appear to suggest that the putrescine reduction is due to either increased putrescine catabolism or greater efflux from the cells, although, again, further work is required if the mechanisms responsible for these changes are to be elucidated. Compound **3** also produced a large increase in AdoMetDC activity (Table 4). This has been observed previously with DFMO and the stereoisomer of E-BED, (*Z*)-1,4-diaminobut-2-ene (Z-BED) (Foster and Walters, 1990; Havis *et al*., 1994a). In the case of DFMO, it was suggested that the increase may be due to stabilization of the active protein as found in animal systems (Foster and Walters, 1990).

Various mechanisms for the inhibition of cell growth by polyamine analogues have been proposed, including inhibition of polyamine biosynthesis by direct enzyme inhibition or regulation of polyamine biosynthetic enzymes (Porter and Sufrin, 1986). Our results would appear to suggest that inhibition of the growth of *P. avenae* was not achieved by direct inhibition of enzymes of polyamine biosynthesis, although an effect via the perturbation of polyamine catabolism or function, for example, cannot be ruled out. A number of bis(benzyl)-polyamine analogues have been shown to inhibit cell growth by repression of polyamine biosynthesis and direct binding to DNA, with subsequent disruption of macromolecular function (Bitonti *et al*., 1989). It would be useful to determine if this is a possible mode of action for the novel cyclic diamines described in this paper.

The novel cyclic diamines described in this work, and the aliphatic and alicyclic compounds described in previous work (Havis *et al*., 1994a,b; Robins and Walters, 1993), exhibit effects on fungal growth that cannot be correlated with depletion of intracellular polyamine concentrations. Further work is necessary to elucidate the mode of action of these compounds and, in particular, to determine whether the observed changes in polyamine levels are related to the antifungal effects of the compounds.

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